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GENETIC POLYMORPHISM IN CASEINS OF COW'S MILK. I. GENETIC CONTROL OF α_s-CASEIN VARIATION

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SUMMARY

Caseins from milk of individual cows were studied by starch-gel-urea electrophoresis. The results indicate that α_s-casein, the calcium sensitive component of α-casein, occurs in at least three major forms. Family studies indicate that this variation is controlled by three allelic autosomal genes with no dominance. Each allele is responsible for the production of one of the three forms of α_s-casein which are designated A, B, and C in order of decreasing mobility. Milk from individual cows may contain any one or any two of these. The six possible types and their corresponding genotypes are: A (A/A), B (B/B), C (C/C), AB (A/B), AC (A/C), and BC (B/C).

Milk samples from 1,378 cows showed the following distribution of α_s-casein types: Ayrshire 98 B; Brown Swiss 192 B, 11 B/C; Guernsey 188 B, 180 BC, 32 C; Holstein 2 A, 81 AB, 5 AC, 410 B, 44 BC; and Jersey 44 B, 21 BC, 2 C. Of 68 crossbreds tested, 67 were B and one was BC. These data do not necessarily reflect the frequencies that would be found in random samples of the breeds. Sire groups and herds were often selected in an attempt to find certain variants.

The reason for the preponderance of the B allele is not apparent. The possibility of some selective advantage is being investigated.

In previous reports (5, 7, 8) it was shown that α_s-casein, the calcium-sensitive component of α-casein, exists in at least three forms. The variants were designated A, B, and C, in order of decreasing mobility. They were discovered by examining caseins isolated from milk samples from individual cows.[1] Separation of the variants was accomplished by the use of starch-gel-urea electrophoresis. The early studies indicated that these variants might be genetically determined, since the first six cows whose milk showed evidence of variation were all daughters of one sire. Since the early reports were published, studies have been directed toward two major objectives: (a) to determine if the variation was genetically controlled, and (b) to find cows that produced only α_s-A, α_s-B, and α_s-C, so that the variants could be isolated for chemical studies. This report presents the results obtained to date in pursuit of these objectives.

MATERIALS AND METHODS

Chemistry. Milk samples were obtained from one milking of each cow. To prepare the casein, skimmilk (gravity separated at 4 C) was precipitated at pH 4.6 and 22-24 C. The precipitate was washed four times each with water and ethanol, twice with acetone, and twice with ethyl ether. Starch-gel-urea electrophoresis, (SGE), as described by Wake and Baldwin (9), was done with casein solutions containing 7 mg casein per ml of buffer-urea solution. Approximately 5 V cm was applied to the gel for 17 hr, after which it was developed with

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[1] During the early part of the investigation a series of whole caseins typed α_s AB and B were sent to Dr. R. Aschaffenburg, National Institute for Research in Dairying, Shinfield, Reading, England. His paper electrophoresis examinations of these caseins disclosed that one AB resolved more clearly than the other AB samples. Dr. Aschaffenburg suggested that this sample was most likely an AC. Further starch-gel-urea electrophoresis studies confirmed this suggestion.

amido black dye. In some of the more recent work, electrophoresis was done with skimmilk diluted with two volumes of buffer to give a solution containing 8 to 10 mg casein per ml. This method (1) also gave good resolution of the α_s-zones and was satisfactory for determining the type of α_s-casein produced by individual cows. Figure 1 shows SGE patterns of individual caseins. Each casein was isolated from the milk of one cow. The six known α_s-casein types are shown.

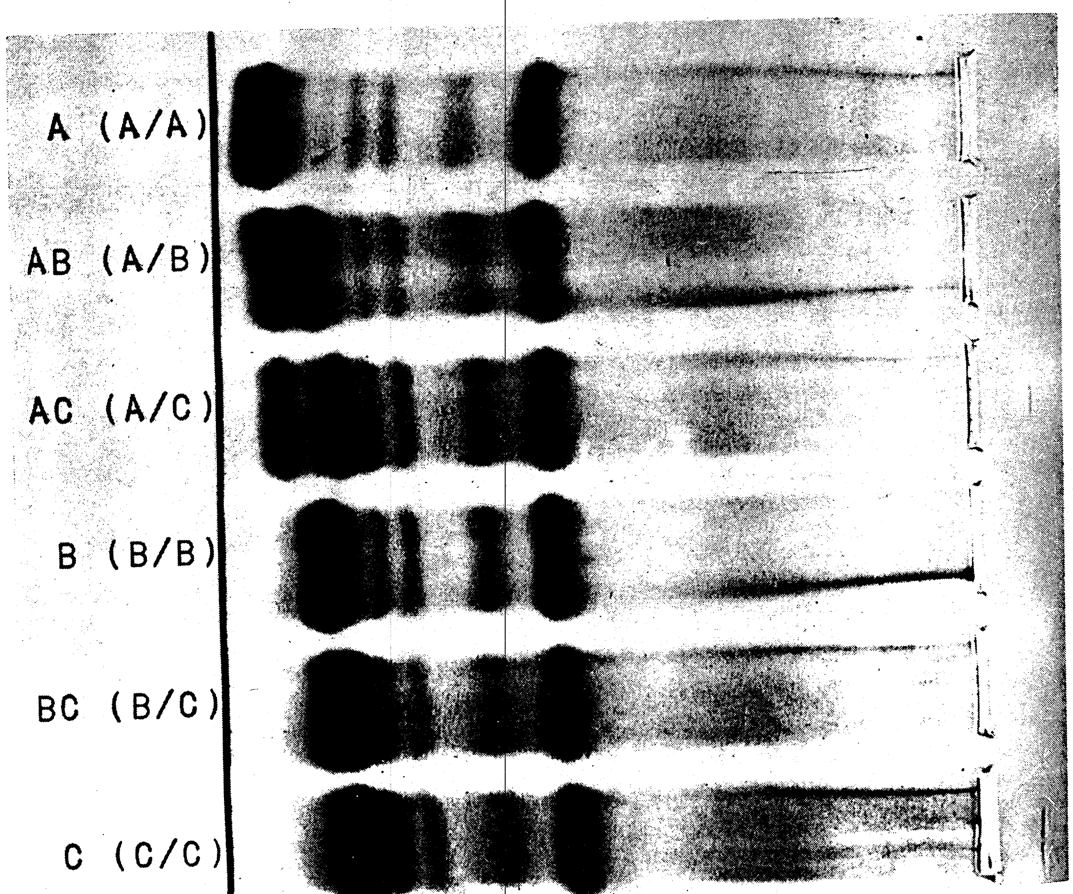

FIG. 1. Starch-gel-urea electrophoresis patterns of caseins isolated from the milk of six individual cows. All of the six possible types are shown with the corresponding genotype designation in parentheses. The slower moving dark band to the right in each pattern is β-casein.

Animals studied. Two approaches were used in selecting animals to be sampled. One was to sample daughters (and their dams where possible) of specific sires, and the other was to sample complete herds. The first animals to be studied were Holsteins, Ayrshire × Holstein crossbreds, and Brown Swiss × Holstein crossbreds from the Beltsville herd. Only six animals in the entire herd had two kinds of α_s-casein (A and B) in their milk. All others had α_s-B only. Since the six AB cows were paternal half-sisters, efforts were made to obtain samples from additional daughters of the sire—a Holstein bull (P-17) used extensively in artificial breeding. One hundred and seven daughters of this bull, and dams of 37 of them, were sampled from herds in Maryland, Michigan, New Jersey, and New York. In addition, 53 daughters of four sons of the P-17 bull were studied. Twenty-nine complete herds were sampled: three Ayrshire, eight Brown Swiss, ten Guernsey, five Holstein, and three Jersey. Three of the herds are in Michigan, one in Pennsylvania, and the rest in Maryland. Milk samples from 1,378 cows were studied.

RESULTS AND DISCUSSION

Table 1 shows the distribution of the various α_s-casein types by breed and crossbreeding

TABLE 1

Occurrence of various α_s-casein types in different breeds

Breed	Total no. of cows tested	α_s-Casein types					
		A	B	C	AB	AC	BC
Ayrshire	98	0	98	0	0	0	0
Brown Swiss	203	0	192	0	0	0	11
Guernsey	400	0	188	32	0	0	180
Holstein	542	2	410	0	81	5	44
Jersey	67	0	44	2	0	0	21
Crossbreds:							
Brown Swiss × Holstein	29	0	28	0	0	0	1
Ayrshire × Holstein	22	0	22	0	0	0	0
Ayrshire × Brown Swiss	17	0	17	0	0	0	0
Totals	1,378	2	999	34	81	5	257
Per cent		0.1	72.5	2.5	5.9	0.4	18.6

groups. It also shows the percentage of animals of each α_s-casein type. It should be pointed out, however, that these data do not necessarily reflect the frequencies that would be found in random samples of the breeds. This is especially true for the Holsteins and, to some extent, for the Guernseys. In both of these breeds efforts were made to sample large numbers of daughters of specific sires. Nevertheless, it is quite apparent that A and C α_s-casein occur much less frequently than B. The α_s-B casein, of intermediate mobility, was found in the milk of 97.0% (1,337) of the cows tested. The α_s-C was found in 21.5% (296) and the α_s-A in 6.5% (88).

The family data indicate that the observed variation in α_s-casein is genetically controlled. These data support the hypothesis that three allelic, autosomal, genes are involved with no dominance. These are designated α_s-Cn^A, α_s-Cn^B, and α_s-Cn^C in accordance with nomenclature suggested by Aschaffenburg for genes controlling variation in β-casein (3).

Analysis of family data is complicated by the fact that the genotype of a bull cannot be determined directly. It must be deduced from study of his daughters and their dams. For example, 106 daughters of the Holstein bull, P-17, were tested, and dams of 37 of these daughters. This was the largest sire group in the study. The next largest was 46. P-17's daughters were distributed by type as follows: 54 AB, 47 B, 4 AC, and 1 BC. Thirty-five of the dams were B and the distribution of types in the 35 daughters was 17 AB and 18 B. It was deduced from these data that this bull's genotype was A/B (α_s-Cn^A/α_s-Cn^B). In A/B × B/B matings, one-half of all the offspring would be expected to be A/B and one-half B/B, and the observed results agree with the expectation. The two other typed dams were A/B and B/C and in each case the daughter was A/B.

Table 2 shows the distribution, by α_s-casein types, of the daughters of bulls whose types have been deduced. Although daughters of 200 bulls were studied, only 60 bulls had enough daughters and dams typed to allow deduction of their genotypes. Naturally, heterozygous bulls were easier to identify than homozygotes. Table 3 shows results obtained from different types of matings. There is excellent agreement with expectation.

TABLE 2

Distribution of daughters by α_s-casein genotypes for different types of bulls

Deduced genotype of bull	No. of bulls	Daughters					
		A/A	A/B	A/C	B/B	B/C	C/C
A/B	4 [a]	2	71	5	68	4	....
B/B	40 [b]	..	4	..	323	12	
B/C	16 [c]	..	..	..	104	110	18

[a] All Holstein.
[b] 5 Ayrshire, 7 Brown Swiss, 4 Guernsey, 22 Holstein, and 2 Jersey.
[c] 12 Guernsey, 3 Holstein, and 2 Jersey.

The ratio of B/B's to B/C's, especially among

TABLE 3

Segregation of α_s-casein alleles in female offspring from certain mating combinations [a]

Mating types [b]		Offspring by α_s-casein genotypes					
		A/A	A/B	A/C	B/B	B/C	C/C
A/B × B/B	Obs.	....	19	....	21	....	....
	Exp.	....	20	....	20	....	....
B/B × B/B	Obs.	....	....	....	88	....	....
	Exp.	....	....	....	88		....
B/B × B/C	Obs.	....	....	....	23	23	....
	Exp.	....	....	....	23	23	....
B/C × B/C	Obs.	....	....	....	12	11	5
	Exp.	....	....	....	7	14	7

[a] Bulls were presumed to have B/B genotype if they consistently transmitted only the α_s-Cn^B gene. They were designated heterozygous if they transmitted both alternative genes.
[b] Reciprocal matings.

daughters of B/C bulls, seems high. Most of the B/C bulls were Guernseys. Only if all dams to which B/C bulls had been mated were B/B (Table 1 indicates they probably were not) would the B/B offspring class be expected to equal the B/C, as it almost does. Analysis of the segregation results from matings between animals of known or deduced (bulls) genotype failed to indicate a significant preponderance of B/B offspring ($X^2 = 3.0$, $P = .22$). The high ratio of B/B's to B/C's was almost entirely due to one sire group in one herd. The sire in question (Genotype B/C) had 46 typed daughters—28 B/B, 15 B/C, and 3 C/C. Twenty-seven of the daughters (21 B/B's, 5 B/C's, and 1 C/C) were in one herd. Dams of eight of these daughters were B/B. Four of the daughters were B/B and four were B/C. This agrees exactly with expected if the bull is B/C. However, considering all daughters, there seems to be an excess of B/B's.

The significance of these findings is obscure at present. It was not possible to obtain enough information from the herds involved to even suggest whether B/B individuals had any selective advantage. In future studies of daughters of B/C bulls, attempts will be made to sample all daughters that calve.

Some of the bulls found to transmit α_s-Cn^A were known to be transmitters of congenital porphyria which may be a recessive defect (6). A connection between the two traits was suspected, but α_s-A was not found in the milk from eight animals affected with porphyria and four known carriers.

These results bring to three the number of milk proteins known to have genetically controlled variants. Aschaffenburg showed that variants in β-lactoglobulin and β-casein were genetically controlled (2-4). The present work indicates that variants in α_s-casein are controlled in the same way. Systems of multiple alleles without dominance have been postulated as the genetic mechanisms controlling all three proteins.

The over-all gene frequencies in the data collected to date are α_s-Cn^A 3.0%, α_s-Cn^B 85.0%, and α_s-Cn^C 12.0%. As pointed out previously, the estimates for α_s-Cn^A and α_s-Cn^C are thought to be high, since many of the animals sampled were selected with a view to finding more of these variants. Nevertheless, the extremely high proportion of α_s-Cn^B is of interest. Is this due to relatively recent mutations, selective advantage for the α_s-Cn^B gene, or a combination of the two? This question cannot be answered at present, but the fact that α_s-Cn^C has been found in four different breeds indicates that the mutation that produced it probably occurred many decades ago. Further speculation could be made in regard to ancestry of the various breeds, but it seems prudent to await further results. Linkage studies in relation to other simply inherited traits are also planned, as well as chemical studies of the proteins themselves.

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